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A TECHNIQUE FOR SANDISON-CLARK EAR CHAMBERS: MANUFACTURE, ASSEMBLY, AND PHOTOMICROGRAPHY

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Abstract

Difficulties for the research worker wishing to use the rabbit ear chamber technique are due in large measure to the insufficiency of published technical information. The present paper is an attempt to overcome this situation and presents—(*a*) a technique for the manufacture of ear chamber components and their assembly, (*b*) some modifications of a technique for operative installation, (*c*) design of a rabbit box suitable for ear chamber work, and (*d*) a method of 35 mm. photomicrography suitable for the rabbit ear chamber.

Introduction

The rabbit ear chamber technique has proved a useful tool in the microscopic study of living mammalian tissues under favourable optical conditions. Examples of different types of chambers which have been used appear in the literature (Sandison 1924; Clark and Clark 1932; Ahern, Barclay and Ebert 1949; Williams and Roberts 1950; Robertson 1951; Williams 1954). As well as connective tissue, Williams (1954) has shown that a variety of tissues grafted into the ear chamber may be used for detailed microscopic study. Observations can be made over extended periods under conditions approximating normality for rabbit tissues.

The limited use of the technique is due partly to its time-consuming character and also to the problems associated with production of the chambers. While the former is common to many worthwhile methods of study, the latter is aggravated by the lack of published details of simple manufacturing methods. Again, in the literature information of the applications of photomicrography to this field is limited.

This paper is intended primarily as a guide to the research worker who, before applying ear chambers to his work, must first deal with the problems of their production. The present account deals only with the particular chamber used in this department. However, once the appropriate techniques have been acquired, modifications could be developed for particular applications.

For accuracy and succinctness of statement, several technical (and even colloquial) terms employed by machinists are used. Where there is doubt as to meaning, any competent technician could provide a 'translation'.

Design and Manufacture of Chamber Components

The Sandison-Clark ear chamber makes possible the microscopic study of living connective tissue which has grown between two transparent plates. Pl. IV, fig. 1 illustrates the arrangement of the chamber parts in relation to the ear tissues.

The ear chamber described is of the round table type (Clark et al. 1930) being made from perspex and mica with perspex pins following the principle of a chamber designed by Ahern, Barclay and Ebert (1949).

The chamber consists of a perspex base with a central raised 'table', 3 threaded perspex pins, a perspex and mica top and 3 brass nuts (Pl. IV, fig. 2). Chamber dimensions are as follows:

External diameter	.. ..	1·200 in.
Diameter of central table	..	0·235 in.
Height of central table	..	0·045 in.
Diameter of perspex pins	..	0·125 in.

The base and top are made from 1/8 in. and 1/16 in. perspex sheet respectively and the pins from 1/8 in. diameter perspex rod.

Chamber Bases

Pieces of perspex, 2 in. square, are sawn from 1/8 in. perspex sheet, retaining the paper on the sheet to prevent surface scratches. Using a 2 in. square metal template (Pl. IV, fig. 3), holes are drilled in each corner of the 2 in. perspex squares. A 3 in. wooden base block is used as a jig to hold the perspex whilst machining. This jig is aligned and held by the 3 jaw chuck of a standard workshop lathe (Pl. IV, fig. 4). 4 small wood screws are used to attach the perspex sheet to the jig for machining. It is advisable to wear protective glasses at all times when working in the machine shop.

A knife tool of left-hand shape with cutting edges on both sides and negative 'top rake' is set in the tool holder so that the right-hand cutting edge is at right angles to the perspex and centred on the work (Pl. IV, fig. 5).

The tool is advanced so that a disc of approximately the desired outer diameter of 1·2 in. is cut out of the perspex sheet. The diameter of this disc is measured accurately by means of a screw micrometer. It is then possible to set the micrometer collar on the cross slide so that, from the next 2 in. square of perspex, another trial disc having the nominal diameter of 1·2 in. is obtained.

Having determined the correct setting, another 2 in. perspex square is screwed to the jig. With the cross slide micrometer set for the correct outer diameter, the lathe carriage is locked with the carriage lock screw and the tool advanced slowly until it just touches the perspex surface. Noting the micrometer reading of the compound slide, the tool is advanced 0·043 in. with the lathe in motion. The tool is then moved across the face of the work 0·4825 in. removing perspex and leaving a central table of 0·235 in. diameter. Returning to the outer diameter position, the tool is advanced 0·002 in. and a finishing cut is taken across to the central table. Again the tool is returned to the outer diameter position and advanced slowly to part the chamber base from the perspex sheet. After this operation the outside diameter of the base is checked for the correct outer diameter of 1·2 in. Having ascertained the correct micrometer collar readings, it is a simple matter to produce a batch of chamber bases.

Chamber Tops

For the chamber tops the dimensions of which are shown in Pl. IV, fig. 6, 2 in. squares are sawn from 1/16 in. perspex sheet and the exact thickness of each square measured and noted. Tops are machined with the same jig, lathe tool and tool holder setting as used for the bases. After setting the cross slide micrometer for the outer diameter the tool is advanced until it just touches the perspex surface, moved across the face of the work 0·225 in. and slowly advanced the measured thickness of the sheet less 0·020 in. After this operation the tool is moved

further across the face a distance of 0·125 in. before being advanced slowly 0·020 in. thus removing a disc of perspex from the middle of the perspex sheet. The tool is withdrawn and the cross slide micrometer collar set for the outer diameter of 1·2 in. before parting the top from the rest of the sheet.

Drilling of the bases and tops now takes place with the aid of a drilling jig.

Manufacture of Drilling Jig

The drilling jig (Pl. IV, fig. 7) is turned from a short length of solid mild steel rod of 1·5 in. diameter. Holding the rod securely in the 3 jaw chuck with 1·5 in. projecting, cuts are taken to reduce the diameter to 0·375 in. over a distance of 1 in. measured from the projecting end. The steel rod is removed from the chuck and the 3/8 in. shaft fitted into the 3/8 in. collet attachment of the lathe. Machining of the other end of the rod now proceeds to reduce its diameter to 1·388 in. and then cuts are taken across the 1·388 in. diameter end section to reduce its axial length to 0·205 in.

A right-hand knife tool is now set with the cutting edge at right angles to the end face of the work and the tool advanced until it just touches the surface. Having noted the micrometer collar reading and beginning at the centre of the work, the tool is advanced 0·080 in. Lateral cutting now proceeds until the internal diameter of the recess thus formed measures precisely 1·202 in. After painting the recess with engineers' blue, and allowing for 'back lash', the tool is moved back towards the centre for 0·101 in., advanced 0·001 in. and the chuck rotated once by hand. This procedure scribes a circle of radius 0·5 in. The tool is then centred, advanced 0·055 in. and moved laterally for 0·125 in. This further recess allows clearance for the central tables of the bases when these are being drilled.

Using a pair of fine engineers' dividers opened to 0·5 in. the scribed circle is divided into 6 equal parts. With the jig held firmly in a vice and using a sharp centre punch, the 6 points on the circle are marked more heavily to expedite drilling. Holding the 3/8 in. shaft of the jig vertically by means of a 3 jaw chuck and using a No. 30 drill, 0·1285 in. diameter (preceded by a somewhat finer drill), holes are drilled at alternate marks on the scribed circle. These holes act as a guide when drilling the bases for the perspex pins. Using a No. 43 drill (0·089 in. diameter) at the alternate marks on the circle, holes are drilled to act as guides when drilling the chamber tops.

Drilling the Chamber Bases and Tops

A base is fitted into the drilling jig with the central table projecting into the central recess, thereby preventing the table top from being scratched. Positioning the jig under the drill press and with the larger holes as guides, 3 holes are made using a No. 30 drill (Pl. V, fig. 8). Similarly, a top is completed by inserting it in the jig and, using the smaller holes as guides, making holes with a No. 43 drill.

Manufacture of Perspex Pins

The pins (Pl. V, fig. 9) are made from 1/8 in. perspex rod which is fitted into the 1/8 in. collet attachment of the lathe. For a distance of 5/32 in. from the projecting end, the diameter is reduced to 0·087 in. During this operation, the parting tool is held at right angles to the axis of the rod to ensure a square shoulder on the pin. Employing a suitable lubricant, the rod with the reduced diameter is threaded with an 8 B.A. die. The pin is then parted from the rod to give a total

length of 13/32 in. (10 mm.). If these pins are required subsequently as a means of holding the chamber on the microscope stage, it may be convenient to allow a total length of 17/32 in. (13 mm.). Brass 8 B.A. nuts are employed to secure the chamber tops to the pins.

Assembly of Chambers

Attachment of Pins to Base

Pins are fitted into the holes in the base so that the shoulder of each pin projects 0·039 in. (1 mm.) above the top surface of the base. Using a fine brush (00, sable) dipped in a perspex solvent such as methylene chloride, the junction of pin and base is touched with the tip of the brush (Pl. V, fig. 10). The solvent runs between pin and base and after a few minutes establishes a firm seal. Pins attached in this way can be removed at a later date by moderate pressure.

Attachment of Mica Cover to Top

Mica covers are made from clear mica sheet approximately 0·004 in. thick. Taking care to avoid scratching the mica, a disc of 0·875 in. (7/8 in.) diameter is cut from the sheet with scissors or a wad punch. This disc is attached to the underside of the perspex top by means of perspex cement, the latter being applied so as to overlap the edges of the disc (Pl. V, fig. 11).

Manufacture of Perspex Buffers

The buffers or 'spacers' stand on the central table and serve to regulate the thickness of the connective tissue which will grow across the table top. To observe cellular detail, thin buffers (20-30μ) are necessary in order to obtain a sufficiently thin layer of tissue. Very thin buffers can be cut from perspex sheet (Ebert, Florey and Pullinger 1939) which is formed by pouring thin perspex solution on to a clean sheet of plate glass laid at a slight incline. After 24 to 48 hours the sheet is peeled off easily and checked for thickness with a metric micrometer. The appropriate area of thin sheet is selected and small square or oblong pieces cut with the aid of a scalpel blade. These pieces are transferred to the correct position on the table top by means of a pointed probe. Usually, buffers of equal thickness are placed equidistant from one another at the periphery of the central table. They are fixed in position by lightly touching the tip of a fine brush dipped in methylene chloride against the side of the central table opposite each buffer (Pl. V, fig. 12). This allows a small quantity of methylene chloride to be attracted in between buffer and table top, and the buffer is soon fixed. It is useful to keep a record of the thickness of the particular buffers employed.

The top can now be placed over the threaded portion of the perspex pins and screwed down with 8 B.A brass nuts to complete the assembly.

Completed chambers should be washed carefully in warm soapy water to remove all dust and grease. Afterwards it is advisable to store them in some suitable container to prevent gross contamination with dust and bacteria (Pl. V, fig. 13).

Operative Installation of Chambers

In general, the methods follow those described by Ebert, Florey and Pullinger (1939) and only modifications will be described.

On account of their increased thickness and greater length of ear, the most suitable rabbits are the lop-eared and semi-lop-eared types. However, any rabbit having an ear length of some 5 in. may be used successfully.

The fur on a rabbit's ears is cut short with electric clippers. The ear is prepared by swabbing with 1:500 Zephiran in 70% alcohol and the chambers and perspex template are sterilized by immersion in 1:1000 aqueous Zephiran for 24 hours.

The following instruments are employed (Pl. V, fig. 14):

(1) mallet;
(2) 2 pairs of fine scissors, one pair having a fine cutting edge ground on the outer side of each blade;
(3) 2 pairs of dissecting forceps, one coarse, one fine;
(4) wooden block, about 2½ in. high, to support ear;
(5) perspex template (Ebert et al. 1939);
(6) straight sided ear punches (Ebert et al. 1939);
(7) 2 scalpel handles fitted with Paragon 17 and 18 blades (Van den Brenk 1956).

Separation of skin from underlying connective tissue and cartilage is aided considerably by use of the fine scissors with a cutting edge ground on the tip and outer side of each blade. These enable one to work more rapidly and with less risk of damage to the larger blood vessels. When the dissection is completed and the chamber is being fitted to the ear, it is advisable to ensure that some fluid blood lies on the central table As the chamber top is screwed down, the correct thickness of blood film is indicated when the buffers on the table top, being pressed against the mica cover, become clearly outlined by the blood film. The shoulders on the perspex pins prevent the vessels of the connective tissue from being compressed.

Rabbit Box

It is convenient to have the rabbit in its natural sitting position in a box which allows an ear to be arranged on the microscope stage. For this purpose a wooden box constructed with a front end slide to take the rabbit's neck, following the principles described by Essex (1948) and Van den Brenk (1956), is satisfactory (Pl. VI, fig. 15).

Photomicrography

Because in photomicrography even small movements give rise to blurred images, for sharp pictures of the tissue in ear chambers it is necessary to employ very short exposures, one thousandth of a second or less. For this reason an electronic flash tube is essential as a light source. In the case of the particular flash unit employed here, the Mannesmann Multiblitz Micro, the flash tube is centred directly under the condenser (Pl. VI, fig. 16). The flash tube housing has upper and lower openings to transmit light from the standard microscope lamp so that direct observations can still be made with the flash tube in its central position. For correct exposure, variation of light intensity is made by interposing neutral density filters between the flash tube and the condenser.

The condenser, fitted to a Leitz stand B (monocular-binocular) microscope, is of the 2 diaphragm type (after Berek) with centring screws. For photomicrography with low power objectives (3·5x, 10x), even illumination is obtained by displacement of the top condenser lens, fully opening the upper diaphragm and

stopping down the lower diaphragm to an optimum in order to increase contrast and depth of field. For photomierography with higher power objectives (24x, 45x), the top eondenser lens is oiled to the base of the chamber and the top (iris) diaphragm adjusted to give the best eompromise between contrast and vertical resolution on the one hand and lateral resolution on the other. For the above condenser, the optimum occurs when the iris aperture is set at about 2 mm. and the field diaphragm aperture set at about 3 mm. The ehamber is held firmly on the stage in a chamber holder, the position of which is adjusted by the mechanieal stage eontrols. Such a holder has been described by Sanders, Dodson and Florey (1954).

Satisfactory photomicrographs are obtained using a 35 mm. eamera whieh is more eonvenient than a quarter-plate camera. A satisfactory arrangement is a Leica 1F camera attached to the single tube of the microscope by means of the Leitz 'Mikas' attachment which employs a prism for continuous viewing. Because of the very elear image obtained through the prism, consistent aecuracy in fine focussing is possible prior to exposing the film. With a reflex camera employing a ground glass screen consistent focus was not obtained.

Thin emulsion fine grain films give better definition and their contrast is valuable when photographing unstained material (Pl. VI, figs. 17-18). Satisfactory films are Ilford 'Micro Neg Pan' and Kodak 'Microfile'. Use of a Hydroquinone caustie developer is advantageous for improving contrast.

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References

Ahern, J. J., Barclay, W. R., and Ebert, R. H., 1949. Modifieations of the rabbit ear ehamber teehnique. *Science* 110: 665.

Clark, E. R., Kirby-Smith, H. T., Rex, R. O., and Williams, R. G., 1930. Recent modifications in the method of studying living eells and tissues in transparent ehambers inserted in the rabbit's ear. *Anat. Rec.* 47: 187-211.

Clark, E. R., and Clark, E. L., 1932. Observations on living preformed blood vessels as seen in a transparent ehamber inserted into the rabbit's ear. *Amer. J. Anat.* 49: 441-77.

Ebert, R. H., Florey, H. W., and Pullinger, B. D., 1939. Modification of a Sandison-Clark ehamber for observation of transparent tissue in the rabbit's ear. *J. Path. Bact.* 48: 79-94.

Essex, H. E., 1948. Transparent ehamber teehnique. 'Methods in Medieal Research' (The Year Book Publishers, Ine., Chieago) 1: 139-45.

Robertson, J. S., 1951. Some developments in rabbit ear chamber techniques. *Aust. J. exp. Biol. med. Sci.* 29: 393-99.

Sanders, A. G., Dodson, L. F., and Florey, H. W., 1954. An improved method for the produetion of tubereles in a ehamber in the rabbit's ear. *Brit. J. exp. Path.* 35: 331-37.

Sandison, J. C., 1924. A new method for the mieroscopie study of living growing tissues by the introduetion of a transparent chamber in the rabbit's ear. *Anat. Rec.* 28: 281-87.

South Bend Lathe Works, 1954. 'How to Run a Lathe.' (South Bend, Indiana, U.S.A.)

Van den Brenk, H. A. S., 1956. Personal communication.

Williams, R. G., and Roberts, B., 1950. An improved tantalum chamber for prolonged microscopie study of living cells in mammals. *Anat. Rec.* 107: 359-73.

Williams, R. G., 1954. Mieroscopie studies in living mammals with transparent chamber methods. 'International Review of Cytology' (Academic Press Ine., New York) 3: 359-98.

Explanation of Plates

Plate IV

Fig. 1—Cross-section through ear chamber in rabbit's ear showing relation of chamber to tissues of the ear.
Fig. 2—Assembled chamber shown alongside chamber components.
Fig. 3—Metal template used to drill perspex squares.
Fig. 4—A wood block held on the jaws of the lathe chuck.
Fig. 5—Showing the shape and position of the cutting tool used in machining the chamber bases and tops.
Fig. 6—Cross-section of chamber top showing dimensions.
Fig. 7—Diagram showing dimensions of jig used for drilling holes in chamber bases and tops.

Plate V

Fig. 8—Drilling jig ready for use under drill press.
Fig. 9—A completed perspex pin.
Fig. 10—Attaching perspex pins to base using perspex solvent and a fine brush.
Fig. 11—Attaching mica window to perspex top by overlapping mica with perspex cement.
Fig. 12—Attaching buffers to central table using perspex solvent and a fine brush.
Fig. 13—Completed chambers and components in a dust free box.
Fig. 14—Instruments used in operative installation of ear chambers.

Plate VI

Fig. 15—Diagrams showing dimensions of rabbit's box. S is a slide which holds the rabbit's neck to one side of the box, G is the groove in which the slide moves, L is the hinged lid.
Fig. 16—Rabbit with ear chamber, microscope with camera and electronic flash tube centred under condenser, as arranged for photomicrography.
Fig. 17 and 18—Photomicrographs of living connective tissue in the rabbit ear chamber. (Fig. 17 × 450, Fig. 18 × 600.)